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An evaluation of Rappaport's magnesium chloride/malachite green medium in the routine examination of faeces

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Rappaport, Konforti & Navon (1956) described a new medium for the isolation of salmonellas from infected material. This medium they claimed to be more effective in isolating salmonellas from faeces than selenite F broth or tetrathionate broth, provided that the inoculum was small. Three or four drops of a 1/1000 saline suspension of faeces in 5 ml. of the medium with subculture after 18 hr. at 37° C. on deoxycholate citrate agar was recommended. A larger inoculum was shown to yield fewer salmonellas.

In order to assess the efficacy of the new medium for routine use, a trial was carried out on faecal specimens in a laboratory which receives for examination material from a general hospital, general practitioners and the public health authorities. Deoxycholate citrate agar, selenite F broth and Rappaport's medium were inoculated in parallel when culturing all faecal specimens received for a period of one year.

METHODS

Deoxycholate citrate agar plates were prepared according to the method of Hynes (1942) and selenite F medium according to Leifson (1936). Rappaport medium was prepared as in the original formula (Rappaport *et al.* 1956) with the exception that magnesium chloride A.R. was used instead of magnesium chloride C.P. A noteworthy point is that in making Rappaport solution B the addition of 40 g. magnesium chloride to 100 ml. of water results in a total volume of 120 ml., giving a concentration of 33·3 %. The complete medium contains 10 ml. of solution B in 113 ml. This gives a final concentration of 2·9 % magnesium chloride and not 4·0 % as stated in the original paper.

Deoxycholate citrate agar was inoculated direct with a loopful of faeces. Approximately 0·5 g. of faeces was added to 10 ml. of selenite F broth, and 10 ml. of Rappaport medium was inoculated with 5 drops of a 1/1000 suspension of faeces in saline. After overnight incubation at 37° C., the fluid media were sub-cultured on deoxycholate citrate agar and incubated for a further 24 hr.

Non-lactose-fermenting colonies were picked and inoculated on urea agar slopes. Urease negative organisms were checked for agglutination with salmonella and shigella agglutinating sera supplied by the Standards Laboratory of the Public Health Laboratory Service. Biochemical properties were confirmed by using diagnostic plates prepared according to the formula of Knox (1949).

RESULTS

A total of 2476 specimens were examined and 99 isolations of salmonellas and shigellas were made, comprising 67 salmonellas and 32 shigellas.

Table 1 shows an analysis of salmonella isolations from the three media. Isolations from Rappaport medium were 91 % of the total compared with 37 % from direct plate culture and 78 % from selenite F. The number of salmonellas isolated from a combination of direct plating and selenite F enrichment was 53. The addition of Rappaport medium raised the total to 67. This represents an increase of 26 % in the number of salmonella isolations.

Table 1. *Salmonella isolations*

Total no. of isolations	Medium	No. of isolations	Percentage of total isolations
67 salmonellas	Deoxycholate citrate agar	25	37
	Selenite F broth	52	78
	Rappaport medium	61	91

No. of salmonella isolations excluding those from Rappaport culture = 53.

Table 2 shows in detail the distribution of positive cultures among the three methods of culture employed for each of the salmonella serotypes encountered. The last column represents the serotype distribution of the 13 isolations which would have been missed if Rappaport's medium had not been included. Omission of selenite F would have resulted in four fewer isolations.

Table 3 shows the isolation rate for shigellas in the same series. Of the 32 isolations only 12 (27 %) were recovered from Rappaport compared with 25 (78 %) both from selenite F and by direct plate culture. None of the positive Rappaport cultures was negative by the combination of the other two methods.

DISCUSSION

Rappaport *et al.* introduced their new enrichment medium to improve salmonella isolations from faeces, as they claim it allows unrestricted growth of salmonellas but inhibits the growth of coliform organisms. Collard & Unwin (1958), in a cultural survey of 1000 faecal specimens at Ibadan, raised the number of salmonella isolations from 16 to 26 by adding a single tube of Rappaport medium to their culture regime. This represents a 60 % improvement in salmonella isolations compared with 26 % in the series reported here, but since the two series are of unequal size comparison is difficult. Sen (1964) also employed Rappaport medium for faecal specimens and increased salmonella isolations by 14 %. It is clear, therefore, that the medium is of value.

Iveson, Kovacs & Laurie (1964) showed the new medium to be of great value in the isolation of salmonellas from contaminated coconut and demonstrated its superiority over both selenite F and tetrathionate broth.

No claim for efficacy in shigella isolation has been made with Rappaport medium. From the present series it appears that *Shigella sonnei* is inhibited by

Table 2. *Salmonellae and the media from which isolations were made*

Serotype	Total	Rappaport + selenite + direct D.C.A. +	Rapp. + sel. + D.C.A. −	Rapp. − sel. + D.C.A. −	Rapp. − sel. − D.C.A. +	Rapp. + sel. − D.C.A. +	Rapp. − sel. + D.C.A. +	Rapp. + sel. − D.C.A. −
Salmonella typhimurium	39	12	13	3	1	1	1	8
S. paratyphi B	3	—	2	—	—	—	—	1
S. heidelberg	1	—	1	—	—	—	—	—
S. reading	4	3	1	—	—	—	—	—
S. thompson	6	1	2	1	—	—	—	2
S. bareilly	1	—	1	—	—	—	—	—
S. bovis morbificans	8	4	3	—	—	—	—	1
S. chester	2	2	—	—	—	—	—	—
S. braenderup	3	—	2	—	—	—	—	1
All serotypes	67	22	25	4	1	1	1	13

Table 3. *Distribution of* Shigella *isolations*

Type	Total	Rappaport + selenite + direct D.C.A. +	Rapp. − sel. + D.C.A. −	Rapp. − sel. + D.C.A. +	Rapp. − sel. − D.C.A. +	Rapp. + sel. − D.C.A. +
Sh. sonnei	30	9	7	8	4	2
Sh. flexneri	2	1	0	0	1	0

the medium. Seven of the 32 shigellas were isolated from selenite F and not from direct D.C.A. plating. This is near the upper limit of the generally accepted improvement to be expected but may be due to the inclusion of some post-treatment specimens in the series. A further seven were isolated from direct plating but not from selenite F. Our shigella isolations were predominantly *Sh. sonnei*. No information concerning the efficacy of the various cultural methods for *Sh. dysenteriae*, *Sh. flexneri*, or *Sh. boydi* was obtained.

It is interesting to note that in the series described here the number of salmonellas isolated from direct plating on deoxycholate citrate agar is very low when compared with Rappaport medium or selenite F, especially as it was introduced as a selective medium for these organisms. Not only does deoxycholate citrate inhibit the coliform group but also to a lesser extent salmonellae. It could be argued that D.C.A. is too inhibitory for general use and it is an impression in this laboratory that efficient plating on MacConkey's agar medium yields as many pathogens, if not more, than on D.C.A. Two and a half times as many salmonellas were isolated after Rappaport enrichment and twice as many after selenite F than on direct plating. Our results seem to indicate that if salmonellas alone are being sought in faeces, primary plating on a solid medium is unnecessary if the two methods of fluid enrichment culture are employed. In the present survey only one of the 67 salmonellas would have been missed if direct plating had been omitted.

A factor which may have influenced the results of this series is that the size of the inoculum varied with each method, but the inocula were similar to the original recommendation in each case. Thomson (1954) and Armstrong (1954) showed an advantage in using small inocula in the plating of faecal specimens. The importance of using a dilute suspension for the magnesium chloride/malachite green medium has been emphasized by Rappaport *et al.* and the employment of this method might have had a bearing on our results.

SUMMARY

Rappaport's magnesium chloride/malachite green medium was employed in the cultural examination of 2476 faecal specimens in parallel with selenite F broth enrichment and direct plate culture on deoxycholate citrate agar. The use of the medium increased the salmonella isolation rate by 26% over the number of isolations from the other two methods, but proved of no additional value in the isolation of shigella organisms. The addition of Rappaport medium is recommended in routine faecal examination.

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